Fibroblast Growth Factor Receptor 3 Is Overexpressed in Urinary Tract Carcinomas and Modulates the Neoplastic Cell Growth

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ABSTRACT

Purpose: Fibroblast growth factor receptor 3 (FGFR3) mutations have been associated with achondroplastic syndromes and urinary bladder carcinomas. Here we describe changes in FGFR3 mRNA and protein expression in transitional carcinomas and determine the effect of monoclonal antibodies against FGFR3 in RT-112 cell line proliferation.

Experimental Design: We used microarray tools to evaluate FGFR3 mRNA expression in 22 urinary bladder carcinomas at different stages (noninvasive pTa, lamina propria invasive pT1, and muscular invasive pT2) and 7 nonneoplastic tissue controls. FGFR3 protein expression was evaluated by Western blotting in 15 different carcinomas and 3 nonneoplastic controls. Two hundred thirty-seven urinary bladder and renal pelvis carcinomas and 21 negative controls were tested on tissue microarrays by immunohistochemistry. The effect on cell proliferation in the RT-112 bladder cancer cell line of monoclonal antibodies against FGFR3 was also evaluated.

Results: Overexpression of FGFR3 mRNA was found in pTa and pT1 stage carcinomas (fold change >8) and in pT2 carcinomas (fold change >4). Nonneoplastic urinary bladder samples do not express FGFR3 protein. However, 83% of pTa, 100% of pT1, and 50% of pT2 carcinomas expressed FGFR3 as determined by Western blotting. By immunohistochemistry, FGFR3 was positive in 71.4% of pTa, 72% of pT1, and 49.2% of pT2 cases as well as 61.5% of upper urinary tract carcinomas. Proliferation of the RT-112 cell line was inhibited with monoclonal antibodies against FGFR3.

Conclusions: FGFR3 seems to play an important role in transitional cell carcinoma development. Our results suggest that FGFR3 antagonists could be developed as possible therapeutics for treatment of urinary tract carcinoma.

INTRODUCTION

Fibroblast growth factors (FGF) represent a large family of polypeptides that are potent regulators of cell proliferation, migration, and differentiation. Although first discovered in cells of mesodermal origin, some are better considered as true epithelial growth factors.

There are four distinct genes that encode for high-affinity receptors for FGFs (FGFR1, FGFR2, FGFR3, and FGFR4; ref. 1). Recently, a new member of this family (FGFR5) was identified in human and mouse (2, 3). FGF receptors (FGFR) belong to the immunoglobulin-like family of tyrosine kinases, and several variant forms of each FGFR have been seen as a result of alternative splicing, substantially enlarging the family. In the case of FGFR3, alternative splicing of the transcript gives rise to two isoforms, IIb and IIC, which vary in their specificity for FGF ligands. The IIb splice variant encoded by exon 8 of the FGFR3 gene binds FGF1 (acidic FGF) preferentially showing a lower affinity for FGF8 (androgen-induced growth factor) and FGF9 (glial activating factor; ref. 4). It is the only FGFR3 isoform expressed in epithelial cells (5). The IIC variant is more promiscuous and binds a wide range of FGFs, including FGF1, FGF2, FGF4, and FGF9 (6–9). Ligand-mediated receptor activation triggers a signal transduction cascade from the cell surface to the nucleus (10) that is translated in a variety of developmental and physiologic processes related to cell growth and differentiation via RasMAPK and STAT1-p21 signaling pathways (1, 11, 12).

It has been reported that activating mutations in FGFR3 are responsible for several autosomal dominant craniosynostosis syndromes and chondrodysplasias (13). On the other hand, activating mutations could also play an oncogenic role in tumorigenesis because they lead to ligand-independent dimerization and constitutive activation of the receptor (14). Thus, activating FGFR3 mutations have recently been found to be present in patients with uterine cervix carcinoma and multiple myeloma, as well as urothelial carcinomas (13). These mutations in FGFR3 are frequent events in low-grade transitional bladder tumors, in which it has been stated that FGFR3 mutation status is the strongest predictor of recurrence when compared with stage and grade (15).

Although the activating mutations are well described in the literature, little is known about the FGFR3 mRNA and protein expression in clinical samples. Moreover, to the best of our
knowledge the possible consequences of inhibiting FGFR3-mediated signaling in neoplastic cells have not been investigated.

Our aims are to describe the change of FGFR3 mRNA expression in nonneoplastic urinary bladder tissue as well as in different stage carcinomas, to show the presence of FGFR3 protein in different clinical samples, and to determine the effect of monoclonal antibodies against FGFR3 in the proliferation of the RT-112 cell line.

MATERIALS AND METHODS

RNA Expression Analysis Based on Microarrays

Samples. We selected transurethral resection biopsies from control nonneoplastic individuals (7 cases) and from patients with urinary bladder transitional cell carcinoma (22 cases). Nine cases were low-grade noninvasive carcinomas (pTaG1), 7 cases were high-grade carcinomas with lamina propria invasion (pT1G3), and 6 cases were high-grade muscle-invasive carcinomas (pT2G3). Every sample was independently reviewed blind by two pathologists (J.J.G-R. and J.F.V.B.), correctly diagnosed and classified in the Pathology Department in the University Hospital Marques de Valdecilla. Sample collection was done according to the Helsinki protocols. Fresh tissue was immediately frozen in liquid nitrogen after extraction and stored at −80°C until processing. Total RNA from samples was pooled within stages and compared with pooled normal specimens.

RNA Extraction. Total RNA was isolated, using the Trizol RNA isolation method (Invitrogen, Paisley, United Kingdom), and purified with the Qiagen RNeasy Mini Kit spin columns (Qiagen, Alameda, CA). RNA concentration was determined spectrophotometrically and RNA integrity was confirmed by electrophoresis of samples on a 1% agarose gel.

Microarrays. We used the GeneChip Test 3 (Affymetrix, Santa Clara), to check the RNA quality before the expression analysis with GeneChip Human Genome U95A (Affymetrix, Santa Clara), representing 12,000 complete human gene sequences; the FGFR3 gene is represented by the Affymetrix probe set 31805_at. Briefly, the FGFR3-specific probes are sense oligonucleotides, 25 bases long, designed based on either the Unigene Hs.1420 sequence or the GenBank M64347 reference (Table 1).

Target Preparation. Double-stranded DNA was synthesized using the Superscript Choice System (Invitrogen). Typically, 10 μg of total RNA was used in a reverse transcription reaction to synthesize cDNA with a primer containing poly(dT) and T7 RNA polymerase promoter sequences. Double-stranded cDNA was extracted with phenol-chloroform followed by precipitation with ethanol. The cDNA was resuspended in 12 μL of RNase-free water and 5 μL of the double-stranded cDNA was used as a template for in vitro transcription in the presence of biotinylated UTP and CTP to generate labeled antisense RNA. The in vitro transcription reaction was done using the Enzo BioArray high yield RNA transcript labeling kit (Enzo Diagnostics, Farmingdale, NY). Labeled RNA was purified with the Qiagen RNeasy Mini Kit spin columns (Qiagen) and quantified spectrophotometrically.

Array Hybridization and Scanning. Labeled cRNA was fragmented in fragmentation buffer [5 × buffer: 200 mmol/L Tris-acetate (pH 8.1), 0.5 mol/L KOAc, 150 mmol/L MgOAc] and hybridized to the microarrays in 200 μL of hybridization solution containing 10 μg labeled target in 1 × hybridization buffer (0.1 mol/L 4-morpholinepropanesulfonic acid, 1.0 mol/L NaCl, 0.01% v/v Tween 20, 20 mmol/L EDTA) and 0.1 mg/mL herring sperm DNA. Test3 arrays (Affymetrix) were hybridized to check cRNA integrity before running the gene expression arrays. In all cases, glyceroldehyde-3-phosphate dehydrogenase 3/5 ratios were below 2. Samples were then hybridized on Human Genome-U95Av2 Arrays (Affymetrix). This single array represents ~12,000 sequences and is derived from sequence clusters in Build 95 of the UniGene database (sequences in UniGene Build 95 are from GenBank 113 and dbEST/10-02-99). Arrays were placed on a rotisserie and rotated at 60 rpm for 16 hours at 45°C. After hybridization the arrays were washed with 6 × saline-sodium phosphate-EDTA-T (0.9 mol/L NaCl, 60 mmol/L NaH2PO4, 6 mmol/L EDTA, 0.01% v/v Tween 20) at 25°C on a fluidics station (Affymetrix) for 2 × 10 min cycles and subsequently with 0.1 mol/L MES, 0.1 mol/L NaCl, and 0.05% SDS, then air-dried.

<table>
<thead>
<tr>
<th>Probe position in mRNA sequence</th>
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<tbody>
<tr>
<td>3227</td>
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<td>3340</td>
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<tr>
<td>3618</td>
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<tr>
<td>3648</td>
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<td>3720</td>
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</table>

Table 1

| Affymetrix probe set 31805_at, FGFR3-specific probes in Affymetrix HG-U95 |
|-------------------------------|-----------------------------------------------------------------|
| Probe consecutive order | Sequence zone | Probe base sequence | Probe position in mRNA sequence |
| 1                       | 3511                      | 5’TCCAGCTAAAGGTTTATAAG-3’ | 3227 |
| 2                       | 3625                      | 5’ATTTTTGGACTCTCAAGCAAGCTG-3’ | 3340 |
| 3                       | 3633                      | 5’GACCTCAAAAGCAAGCTGTTTTT-3’ | 3348 |
| 4                       | 3663                      | 5’AAATTTTCTTAAATGTGTGTGGG-3’ | 3378 |
| 5                       | 3684                      | 5’TCCAGCCAGGGAGCGTTTCCAG-3’ | 3399 |
| 6                       | 3716                      | 5’CCCGCCTGGTGTCAGGTTGATG-3’ | 3431 |
| 7                       | 3722                      | 5’TGGTGTCAGGTTGAGTTGATTAT-3’ | 3437 |
| 8                       | 3821                      | 5’ACGTCTTACAGGAGTGTGTAGG-3’ | 3536 |
| 9                       | 3825                      | 5’CTTCGAGGCTGTTGAGTGTAGG-3’ | 3540 |
| 10                      | 3831                      | 5’GCAATGCTTCTCTAGTTTATAGGC-3’ | 3546 |
| 11                      | 3861                      | 5’TGCATTCTCTCAAAGCTGGAGGGA-3’ | 3576 |
| 12                      | 3873                      | 5’AAGCTGGAGGAGGAAGCCGTAATCC-3’ | 3588 |
| 13                      | 3891                      | 5’TGAATCTGTTGTTGGTGTGTTGAC-3’ | 3606 |
| 14                      | 3903                      | 5’GGCGGCTTCTCTGTTTTACGGCC-3’ | 3618 |
| 15                      | 3933                      | 5’TCTGAGCGAGCTGTCCTGTGTCG-3’ | 3648 |
| 16                      | 4005                      | 5’GGCCAGAGTGTTGACCAAAGCG-3’ | 3720 |
0.01% Tween 20 at 50 °C for 4 × 15-minute cycles. The arrays were then stained with a streptavidin-phycocyanin conjugate (Molecular Probes, Eugene, OR), followed by 10 × 4 wash cycles. To enhance the signal arrays they were further stained with anti-streptavidin antibody for 10 minutes followed by a 10-minute staining with a streptavidin-phycocyanin conjugate. After 4 × 15-minute additional wash cycles arrays were scanned using a confocal scanner (Agilent GeneArray Scanner, Affymetrix). The scanned image data were analyzed using Microarray Suite 5.0 (Affymetrix).

**Protein Expression Analysis in Tissue Samples by Western Blot**

**Samples.** Three urinary bladder samples from otherwise healthy individuals, six low-grade superficial carcinomas (pTaG1), five high-grade lamina propria invasive carcinomas (pT1G3), and four high-grade muscle-invading carcinomas (pT2G3) were processed for Western blot analysis with antibodies raised against FGFR3. Samples were from different patients to those used for the microarray analysis; however, the histopathologic analysis was done exactly as for the samples used for the microarray analysis. Fresh tissue was immediately frozen in liquid nitrogen after extraction and stored at −80 °C until processing.

**Protein Extraction.** Frozen samples were homogenized and pulverized in liquid nitrogen. Radioimmunoprecipitation assay (RIPA) B buffer [sodium phosphate 20 mmol/L (pH 7.4), NaCl 150 mmol/L, Triton X-100 1% v/v, EDTA 5 mmol/L] was added as well as a protease inhibitor mixture (Complete, Roche Diagnostics Inc., Mannheim, Germany).

**Western Blot.** Protein samples (20 μg of total protein) were mixed with SDS-PAGE gel loading buffer supplemented with 5% of β-mercaptoethanol and incubated at 100°C for 5 minutes before being loaded on 6% polyacrylamide gels. After electrophoresis, proteins were transferred to nitrocellulose membranes. Duplicate gels were run and blotted. One membrane was probed with antibodies raised against the FGFR3 protein (Santa Cruz Biotechnology, Santa Cruz, CA) and the second membrane was probed with antibody raised against actin (Amersham, Little Chalfont, United Kingdom) as a control for protein loading. Finally, membranes were hybridized with a secondary antibody conjugated with peroxidase (Amersham) and the chemoluminescent signal was detected using the enhanced chemiluminescence system (ECL, Amersham) with high-performance chemiluminescence film (Hyperfilm ECL, Amersham).

**Cell Lines**

Tissue culture reagents were obtained from Invitrogen (Paisley, United Kingdom). The RT112 human bladder carcinoma epithelial cell line was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). RT-112 cells were grown in RPMI medium, supplemented with 10% fetal bovine serum (FBS) and 2 mmol/L glutamine, except where otherwise stated.

**Preparation of Protein Lysates.** Cells from a 10-cm plate were washed twice with 1 × PBS (pH 7.4) and collected in 0.5 mL of RIPA B. Samples were centrifuged at 15,000 × g for 10 minutes at 4 °C to pellet cellular debris. The supernatant was kept and the protein concentration was measured using the Bradford protein assay (Bio-Rad, Hercules, CA; ref. 16). Western blot analyses of protein extracts obtained from cultured cells were done as described above.

**Cell Proliferation Assays.** Experiments were done to evaluate the effect of a mouse monoclonal antibody raised against human FGFR3 on proliferation of RT-112 cells by comparing the proliferation rate of cells grown in the presence of the antibody raised against FGFR3 with proliferation in the presence of a control antibody raised against mouse β2-microglobulin (Santa Cruz Biotechnology). The preservative sodium azide was first removed from the antibody solutions by washing and concentrating the antibodies thrice with PBS using a 10-kDa centricon filtration device (10-kDa MWCO, Millipore, Bedford, MA) followed by filter sterilization through a 0.2-μm filter previously saturated with DMEM and 10% FBS. Antibodies were diluted in culture media. RT-112 cells were seeded in a 96-well plate at a density of 2 × 10³ cells per well (0.2 mL) in RPMI medium containing 10% FBS. Cells were allowed to attach to the wells for 24 hours before the RPMI medium was removed and replaced by fresh RPMI, either serum free or supplemented with 10% FBS, containing antibodies at concentrations of 0, 0.02, 0.2, 2, and 20 μg/mL. The cell proliferation rate was estimated after 24 and 48 hours by measuring the formation of reduced MTT (Sigma Chemical Co., St. Louis, MO). Briefly, after 1 and 2 days incubation, media was removed and replaced by 100 μL of 1 mg/mL MTT in RPMI medium containing 10% FBS. To provide the blanks for absorbance readings some control wells of medium alone were included. The plate was incubated for 30 to 60 minutes at 37°C. After the medium was removed, 100 μL of DMSO was added to each well. Cell viability was determined by MTT absorbance at 550 nm and extrapolation of the absorbance intensity from a standard curve.

**Protein Expression Analysis in Tissue Samples Using Tissue Microarrays**

A total of 209 cases of urinary bladder transitional cell carcinomas from transurethral resection biopsies and cystectomy specimens, 28 renal pelvis transitional cell carcinomas, and 21 normal urinary bladder and kidney tissue samples (258 samples in total) were selected from the Surgical Pathology archives of the Hospital Universitario Marques de Valdecilla. All paraffin-embedded donor tissue blocks were sampled with 0.6-mm punchers using a tissue microarray instrument (Beecher Instruments Inc., Sun Prairie, WI). Paraffin tissue array blocks containing arrayed core cylinders from 37 pTa, 100 pT1, 72 pT2, and 28 renal pelvis transitional carcinomas were subjected to routine H&E staining and immunohistochemical staining for FGFR3 protein (monoclonal, dilution 1:25, Santa Cruz Biotechnology). Briefly, antigen retrieval was done by boiling sections in citric acid buffer using a pressure cooker for 90 seconds. The Dako EnVisionTM+ kit (Dako, Glostrup, Denmark) was used as a visualization system according to the manufacturer’s instructions in a Techmate 500-220 automated immunostainer (Biotek, Santa Barbara, CA). Diaminobenzidine was used as the chromogen.

Positive FGFR3 staining was defined as a coarse cytoplasmic membrane reactivity. Immunohistochemistry was considered negative in cases with weak staining of <5% of the cells. To reduce interobserver variation in the evaluation, three
independent pathologists (JFVB, JCG and JJGR) evaluated staining patterns and a consensus score for each section was achieved.

RESULTS

Microarray Results

Analysis of differential expression of the FGFR3 gene in neoplastic samples was done using the Affymetrix microarray data. The following parameters were considered in the analysis: detection (classification of the gene as present, absent, or marginal in each sample); change (indicating an increase, decrease, or no change for each sample); and the signal log ratio (SLR; indicating the change in expression levels between a baseline control and each sample). This last parameter is expressed as log2 ratio (base 2 logarithm of the fold change or number of times that gene expression is higher or lower than nonneoplastic control sample).

Compared with controls, expression levels of FGFR3 were increased more than 8-fold (SLR >3) in pTaG1 (8.57) and pT1G3 (9.85) carcinomas and more than 4-fold (SLR >2) in T2G3 carcinomas (4.79; Table 2).

Protein Expression

Western blotting of biopsy samples with antibodies raised against the FGFR3 protein detected several immunoreactive bands of distinct molecular weights (Fig. 1; Table 2). FGFR3 protein was not detected in the control nonneoplastic samples analyzed; however, FGFR3 was present in 73% of the carcinoma samples examined (11 of 15 cases). Most of the samples positive for FGFR3 expression were low-grade noninvasive carcinomas, whereas 50% of high-grade muscle–invasive carcinomas were positive.

FGFR3 Expression in RT-112 Cell Line

Expression of FGFR3 protein in RT-112 cell was tested by Western blotting and showed the same banding pattern as that observed in carcinoma samples from patients harboring transitional carcinomas (Fig. 2).

Cell Growth Inhibition by Monoclonal Antibodies Raised Against FGFR3

As shown in Fig. 3, antibodies raised against FGFR3 inhibited proliferation of RT-112 cells in serum free media after 48 hours, but anti-β2-microglobulin showed no effect (Fig. 3). On the other hand, in media containing 10% FBS, none of the antibodies showed a significant effect on cellular proliferation (data not shown).

Protein Expression Analysis in Tissue Samples Using Tissue Microarrays

Of the tissues sections that could be reliably evaluated immunohistochemical staining for FGFR3 was positive in 69.4% of pTa, 72% of pT1 and 49.2% of pT2 cases. In addition 61.5%

![Fig. 1](image)

**Table 2** Results of FGFR3 gene (Hu95Av2 microarray) and protein (Western blot and tissue microarray) expression

<table>
<thead>
<tr>
<th></th>
<th>Nonneoplastic control</th>
<th>pTa carcinoma</th>
<th>pT1 carcinoma</th>
<th>pT2 carcinoma</th>
<th>Transitional cell carcinoma, renal pelvis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affymetrix-based mRNA expression (SLR)*</td>
<td>Absent</td>
<td>Present (3,1)</td>
<td>Present (3,3)</td>
<td>Present (2,26)</td>
<td>NA</td>
</tr>
<tr>
<td>Western blot (%)</td>
<td>0/3</td>
<td>5/6 (83%)</td>
<td>3/3 (100%)</td>
<td>3/6 (50%)</td>
<td>NA</td>
</tr>
<tr>
<td>Tissue microarray (%)</td>
<td>1/20 (5%)</td>
<td>25/36 (69.49%)</td>
<td>67/93 (72%)</td>
<td>33/67 (49.2%)</td>
<td>16/26 (61.5%)</td>
</tr>
</tbody>
</table>

NOTE. Microarray hybridization based on Affymetrix MAS5.0 software. Western blot and tissue microarray results for FGFR3 protein expression.

Abbreviation: NA, not available.

*SLR in parenthesis.
†Positive versus total cases.
‡Urinary bladder nonneoplastic tissue.
§Positive versus valid cases.
∥Renal nonneoplastic tissue.
of the transitional cell carcinomas from the renal pelvis were positive (Fig. 4; Table 2). Only one case of otherwise healthy bladder tissue (<5% of the controls) was positive for FGFR3 and expression was restricted to small foci of cells.

DISCUSSION

FGFs are a family of proteins that bind specifically to cellular surface receptors called FGFRs with ligand-mediated activation of the receptor leading to a variety of developmental and physiologic processes related to cell growth and differentiation. FGFR3 activating mutations are responsible for several autosomal dominant craniosynostosis syndromes, chondrodysplasias (13, 17) and some neoplasms including multiple myeloma (18) and uterine cervical carcinomas (19). Urinary bladder tumors, however, are the most frequently affected and particularly low-grade noninvasive carcinomas. It has even been stated that detection of mutations in FGFR3 is a more sensitive means than cytology for identifying superficial tumors (14, 20) although the same technique fails to detect more advanced carcinomas (21). It is interesting the absence of mutations in the in situ carcinomas analysed (21), and the activating point mutations described in benign urothelial papillomas (22). However, these results are not universal, and other studies have found an important set of in situ carcinomas as well as advanced tumors that displayed FGFR3 mutations (14). On the other hand, the presence of activating point mutations seems to have prognostic significance, with a lower recurrence rate than the cases in which FGFR3 is not mutated (15, 23). We have not evaluate the prognostic significance of FGFR3 overexpression because all of our cases were recent cases with a short follow-up (<1 year).

We have shown a marked overexpression of both FGFR3 mRNA and protein in transitional carcinomas with a greater effect being observed in stages pTa and pT1. Most of the pTa and pT1 carcinomas showed protein overexpression in Western blot experiments and by immunohistochemical staining. Previously reported frequencies of mutations in FGFR3 are approximately 75% in pTa and 20% in pT1 cases (21). Thus, FGFR3 protein overproduction seems to occur more frequently in low grade transitional carcinomas than activating mutations. Among the more severe muscle-invading carcinomas 50% showed elevated levels of FGFR3 mRNA and protein, however, activating mutations have been described in only 16% of T2 carcinomas (21).

From the biochemical point of view, the FGFR family has more than 100 different combinations possible at the cell
surface as well as truncated forms (1). This molecular heterogeneity is also observed in the Western blots, in which our analysis showed bands forming a ladder of different size proteins, probably corresponding to both truncated forms of the protein and to different glycosylation states as previously described (9, 12, 24). Only two phosphorylated intracellular immature forms of FGFR3 (98 kDa and 120 kDa) have been detected in tanatophoric dysplasia cases, highlighting the importance of these proteins as mediators of abnormal signaling in this disease (12). In Western blotting we observe a 130-kDa FGFR3 form in our neoplastic samples. Moreover, we have showed that the protein colocalizes with the cellular membrane by means of immunohistochemistry, an observation that argues against FGFR3 maturation defects in transitional cell carcinomas.

Overexpression of FGFR3 could thus provide a good diagnostic marker for transitional cell carcinoma with a high level of selectivity. Regarding the specificity of FGFR3, again, all the published data are referred to mutation status, and, apart from uterine cervix carcinomas and multiple myeloma (13), no mutations have been detected in upper aerodigestive tract, esophagus, stomach, lung or skin carcinomas (25) in normal and benign prostatic hyperplasia (26) and breast carcinoma (27) suggesting that FGFR3 mutations are very specific to bladder carcinomas. The immunohistochemical data provides the first report of FGFR3 expression in upper urinary tract carcinomas, a fact that could indicate an important role of this receptor in development of urinary tract neoplasias.

Further support for a key role of the FGFR3 protein in the development of transitional cell carcinomas is provided by the strong antiproliferative effect of monoclonal antibodies raised against FGFR3 in the RT-112 cell line. Although this effect was observed only in serum-free media, it is possible that activation of parallel growth signaling pathways was triggered by bovine growth factors present in the serum. To the best of our knowledge this effect has not been described previously and raises the possibility that FGFR3 could be considered as a molecular target for therapy. A recent study of human myeloma cell lines that constitutively express active mutant FGFR3 showed that cell cycle parameters were decreased and the rate of apoptosis increased by FGFR-specific tyrosine kinase inhibitors (28).

In summary, we have described the overexpression of FGFR3 mRNA in a significant number of pTa and pT1 stages of transitional cell carcinomas from the urinary bladder and renal pelvis. Fifty percent of the pT2 advanced urinary bladder carcinomas also showed significant mRNA overexpression. FGFR3 protein was detected in Western blot analysis of

![Fig. 4](image-url)
transitional carcinoma biopsy tissue and in immunohistochemical analysis of tissue microarray, whereas the protein was generally absent (>95%) from the nonneoplastic control samples. The antibodies raised against FGFR3 had a prominent antiproliferative effect in RT-112 cell line culture. Thus, FGFR3 could serve as a molecular marker for urinary bladder transitional cell carcinoma and deserves more investigation into its potential as a target for antineoplastic therapy.

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Correction: Article on Fibroblast Growth Factor Overexpressed in Urinary Tract Carcinomas

In the article on Fibroblast Growth Factor Overexpression in the January 15, 2005 issue of Clinical Cancer Research, Miguel Molina was not included in the list of authors. The correct list of authors is, as follows:


Fibroblast Growth Factor Receptor 3 Is Overexpressed in Urinary Tract Carcinomas and Modulates the Neoplastic Cell Growth


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